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EDITORIAL

Have microfluidics delivered for drug discovery?

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1. Introduction

The drug discovery process is usually initiated with a benchtop discovery in which cultured cells are observed to respond to a drug, which is then tested in animal and human clinical trials prior to bringing a product to market. Drug development expenses in the pharmaceutical industry have skyrocketed in recent years, while the number of truly innovative drugs approved by governmental regulatory bodies is decreasing [1]. Cell-based high-throughput screening (HTS) techniques have enabled researchers to screen over a million compounds in 1–3 months [2], but only 1 in every 5000 promising 'hits' successfully transitions from the benchtop to the market [3], as the vast majority of discoveries fail during animal testing and human clinical trials. This low success rate results in an average development time of 10–12 years, with an estimated average cost of $2.6 billion to bring each drug to market [4], resulting in unsustainable costs to global health-care systems.

Currently, automated HTS methods using robotic handling and analysis systems are the gold standard in drug discovery. The design requirements of these systems seem ideally suited toward applying novel microfluidic approaches to improve HTS. Microfluidics is an established strategy to reduce culture vessel sizes and precisely control fluid movement down to the picoliter range. Miniaturizing and integration of microfluidics with other complimentary microsystems would enable massive parallelization and highly combinatorial cell-based assays, reducing the overall cost of reagents, and improving assay reproducibility, speed, and experimental throughput [5]. These advances should have substantially reduced the cost of drug discovery, while enabling the unique discovery of new therapeutics that would not have been identified using conventional technologies.

Yet, despite rapid development of expertise in designing, fabricating, and operating microfluidic systems over nearly two decades, to the best of our knowledge, no commercially available drugs have been discovered as a unique result of microfluidic technologies. While microfluidics has been adopted into liquid-handling systems that automate the screening process, the assays themselves are still conducted in multi-well plate dishes. The reasons for this are varied. Perhaps discoveries have been made, but are still in the drug development pipeline. More likely, however, is that although these systems are powerful in the lab, they require skilled operators and are not robust or scalable enough to handle the stringent demands for HTS of millions of compounds, while the existing multi-well plate technologies are more than adequate for these needs.

Although several companies (BellBrooks, Dolomite, Caliper, and Nanoscale, among others) have developed high-throughput tools and robust fabrication methodologies to incorporate microfluidics into culture plates, we believe that the real power of microfluidic systems for drug discovery still remains to be realized. In this short opinion paper, we highlight three high-potential research areas for microengineered systems in drug discovery and recent articles that build toward better therapeutic discovery platforms. Specifically, we discuss how microengineered systems may be useful to probe functional activity of cells in HTS, develop more realistic environments within which to screen drugs, and build organ-on-a-chip constructs to prescreen candidate therapeutics.

Cell-based assays have traditionally relied upon some identified biomarker that indicates how well the candidate therapeutic is performing. Although useful, it remains uncertain whether the selected readouts are sufficient to ensure that the treatment is holistically altering cell activity. Hence, analyses of *functional* cellular activity are now being considered as readouts that may have greater predictive potential, and microengineered systems are well suited to measure these activities. For example, microfluidic cytometers have been developed to identify potentially cancerous cells based on changes in the intrinsic stiffness of the cell, which presumably allows them to metastasize through tissue [6]. Cell-generated contractile forces may also signify functional activity tied to homeostasis or disease progression, and measuring these forces may provide an integrative picture of overall cell health. While several techniques exist to measure cellular forces [7–9], these have only recently been adapted for large-scale HTS drug screening by Park et al., who developed multi-well plates with integrated soft hydrogel culture surfaces labeled with fiduciary markers [10]. Contractile force was used to identify novel chemicals from existing libraries that reduced the forces generated by asthmatic airway smooth muscle cells. Several techniques exist to measure cellular forces [11], and so this

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approach may also be useful in repurposing drugs for novel applications [12].

A second strategy to leverage microengineered technologies for drug discovery is in the design of the cell culture environment. The cellular microenvironment plays a critical role in directing cell response to therapies, but these factors are typically ignored in a conventional HTS assay, in which cells are cultured on hard, flat, plastic dishes. Several recent reviews outline a wide variety of microengineering techniques that recreate these environmental factors including environmental mechanics, gradients, and cell-to-cell interactions, with high precision and specificity [13]. However, scaling these technologies for HTS is particularly challenging, given typical success rates in fabrication and platform robustness. Hence, identifying the minimal set of microenvironmental parameters that drive realistic cell activity remains a critical challenge. One such factor that has emerged is the concept of culture dimensionality: it is now well established that three-dimensional (3D) culture systems direct cell behavior in distinct patterns, compared to their two-dimensional (2D) counterparts. However, culturing cells in 3D presents some unique challenges, including a substantial increase in cost of materials for cell culture, greatly increased imaging and analysis time, as well as difficulties in ensuring that therapeutics are transported through the 3D matrix, in a manner similar to transport through vascular networks available *in vivo* [14]. Recent work from our lab has demonstrated an HTS-scalable approach by printing miniaturized 3D cultures in <1 μL volumes. Doing so reduces the additional costs of 3D materials, accelerates imaging time, and minimizes diffusion-based limitations in transport of molecules in nonvascularized tissue. To circumvent evaporation-driven cell damage that usually limits hydrogel miniaturization, an all-aqueous printing technology [15] was developed and used to print 3D breast cancer cultures in a 384-well plate with a robotic liquid handler. An *in vivo*-like reduction of chemotherapeutic activity was demonstrated in the 3D culture systems, suggesting that for an additional $5 per 384-well plate, 3D HTS cultures may be used to better identify anticancer drugs at the discovery stage [16].

An alternative strategy to generating 3D environments is to let the cells generate those environments themselves. Spheroid culture has emerged as an important tool to study cancers in realistic environments, in which cells adhere to each other forming a 3D body. Microfabrication approaches were recently developed by Vrij et al. to scale spheroid culture up toward HTS levels by using a plastic thermoforming process to create dense arrays of microwells within a microplate [17]. The resulting structures allows seeded cells to cluster and form 3D bodies that can then be assayed with a large number of replicates for each drug to be screened. The resulting HTS screen identified novel compounds to direct differentiation. Taken together, these works show that HTS in simplified realistic microengineered environments is possible, and that it may provide important advantages to the drug discovery process [17].

Rather than focusing on improving the quality of the original drug discovery, an alternative strategy to reduce the cost of drug development is to screen out discoveries prior to expensive animal and human trials. Organs-on-a-chip are advanced micro- and tissue-engineered models capable of simulating realistic organ environments, including multicellular architectures, tissue–tissue interfaces, physicochemical microenvironments, and vascular perfusion (Figure 1). By recreating detailed *in vivo*-like conditions, organ-on-a-chip platforms are hoped to predict translational drug efficacy and toxicities. For example, a liver-on-a-chip was engineered to mimic heterotypic cell interactions in primary human hepatocytes, and was used to analyze toxicity of the drug diclofenac [18]. A heart-on-a-chip containing beating cardiomyocyte films was used to quantify the effects of the beta-adrenergic agonist isoproterenol [19]. A lung-on-a-chip was designed to analyze damage to the epithelium by rupture of liquid plugs and to quantify the effects of the clinical surfactant [20]. A bone-marrow chip was developed to screen drugs for radiation safety [21], and a tumor-on-a-chip fabricated to study tyrosine kinase inhibitors [22], among many other examples [23]. Though several organs-on-chips have now been developed, these systems are currently not well suited to certain areas of drug discovery, such as chronic diseases, adaptive immune responses, and complex system-level behaviors of the endocrine, skeletal, or nervous systems.

2. Expert opinion

Microengineered strategies have not as yet delivered for drug discovery, in that they have not, to date, been uniquely used as the critical technology to bring a drug through the discovery and development pipeline. However, new microengineering-enabled strategies may significantly improve the drug development process as outlined in this article, by (i) enabling high-throughput *functional* readouts to predict therapeutic activity, (ii) conducting high-throughput screens for drug discovery in highly realistic engineered culture microenvironments, and (iii) developing organ-on-a-chip platforms to screen out those therapeutics that would ordinarily fail during expensive animal testing and human clinical trials later in the drug discovery pipeline. Critical challenges remain in making these technological advances practically feasible for real-world biological discovery. The optimal design solution for drug discovery is one in which the technology is sufficiently complex and realistic to improve the translational potential and ultimate utility of any discovery, while also being simple enough to allow for assay reliability and operational simplicity. While microfluidic technologies are improving with every design iteration, much work remains to be done before they are reliable and robust enough to deliver on the demanding and stringent requirements for high-throughput screens. Similarly, the technologies must become simple enough to be operated by end users with nonengineering backgrounds and expertise. This is critically important: if an invention is not used by the people for which it is intended, then even the most impressive technological achievements will have only a limited impact on society. Finally, microengineered screening platforms must develop simultaneously with our understanding of biological systems so as to recapitulate those aspects most critical for drug discovery and development. For example, it is becoming apparent that the human body acts as a system, and the traditional reductionist approach in biology does not account for systemic interactional complexities between organs. The combination of recent technological developments and these biologically driven design goals suggests an exciting future for microengineered platforms in drug discovery. The logical

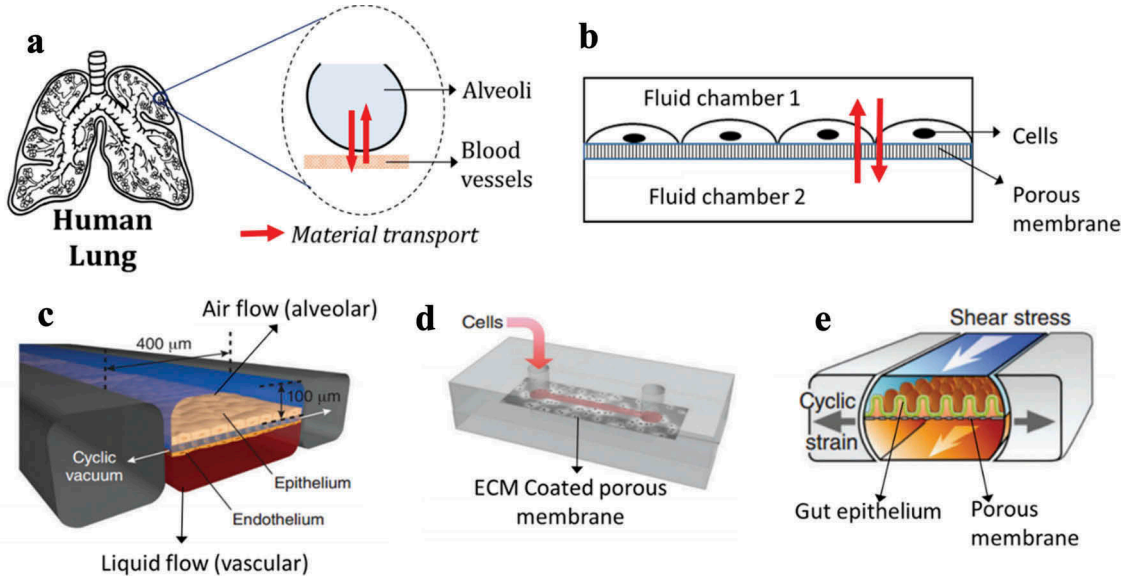

Figure 1. Organs-on-a-chip to pre-screen candidate therapeutics (Figure adapted from [21] with permission of Nature Publishing Group) (a) Cartoon representation of an alveoli unit in a human lung, indicating material transport of nutrients and waste across the lung-blood barrier. (b) Schematic representation of the functional operation of this organ, in which two fluid chambers are separated by a cell-lined barrier, which regulates material transport between the two chambers. This functional structure can then be fabricated on-chip using multilayer microfluidic systems. (c) The human lung translated to a Lung-on- a-chip system to study drug toxicity induced pulmonary edema, with human alveolar epithelial cells cultured on top of a flexible, porous, ECM-coated membrane (upper air channel) and human capillary endothelial cells on the bottom on the vascular channel. Breathing motions are simulated by cyclic suction to full-height side chambers that rhythmically actuates the flexible PDMS side walls attached porous membrane (d) A similar barrier structure also applies to developing a kidney-on-a-chip model for nephrotoxicity assessment, with human kidney proximal tubular epithelial cells cultured on the top of a porous membrane separating two channels, enabling analysis of transcellular transport, uptake and secretion. Similarly, (e) a gut-on-a-chip can be fabricated with intestinal epithelial cells cultured on top of an ECM-coated, porous PDMS membrane separating two channels, and cyclic suction applied to side chambers mimicking peristaltic condition.

extension of the organ-on-a-chip field is to link these organ modules in a realistic fashion to realize a 'human-on-a-chip', and eventually a personalized human-on-a-chip, using a specific patient's own cells to experimentally predict a patient's individualized response to potential therapies. Taken together, advances in drug screening to discover new potential therapies and the development of these hypothetical 'patients-on-a-chip' to pretest any discoveries suggest that microengineered tools and techniques could eventually reduce the substantial cost of drug discovery that burdens global health-care systems today.

Declaration of interest

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